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A Critical Comparison Study on the pH-Sensitive Nanocomposites Based on Graphene-Grafted Chitosan for Cancer Theragnosis

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Abstract

Drug delivery is one of the major issues in the world of science, which receives a large part of the research in various fields. The ultimate goal of drug delivery is to help the patient with developing advanced drug delivery systems. These systems revolutionize the treatment of many diseases including cancer. Effective drug carriers can significantly reduce the undesirable side effects of anticancer drugs through the drug controlled release and using the selective drug to cancerous tissue. The natural biocompatible and biodegradable polymer of chitosan is widely investigated in drug delivery systems, especially as the carrier for anticancer drugs. An important aspect for the application of chitosan in drug delivery for cancer treatment is its pH sensitivity. Graphene attracted much attention in various fields as a shining star in the science of materials. In recent years, the use of graphene in the diagnosis and treatment of cancer is also investigated. Therefore, chitosan-graphene nanocomposite can be introduced as a pH-sensitive carrier for cancer theragnosis. In the current study, the application of chitosan and graphene in the treatment of cancer addressed in previous years was discussed.

INTRODUCTION

Cancer is a life-threatening disease caused by abnormal cell proliferation, and one of the causes of death in developed countries. According to data from the American Cancer Society, 6,000,920 deaths of cancer were recorded in 2017. Prostate cancer, lung and bronchus cancer, and colon and rectum cancer in males and breast cancer, lung and bronchus cancer, and colon &and rectum cancer in females were the most common cancers in 2017, respectively. Lung and bronchus cancer with about 26% had the highest mortality rate among cancers. Table 1 shows the number of people with cancer and cancer deaths in 2017 in the United States. These data were collected by the American Cancer Society [1].

Cancer also threatens the health of children; about 10,270 children aged 1 to 14 years in 2017 were diagnosed with cancer in the United States. Leukemias are the most common cancer among children in which white blood cells have abnormal growth [1].

In Iran, after coronary heart disease and accidents,

Table 1: Number of New Cancer Cases and Deaths of Some Cancers in the United States in 2017

	New Cases, No.		Deaths, No.	
	Male	Female	Male	Female
Lung and Bronchus	105,510	116,990	71,280	84,590
Colon and Rectum	71,420	64,010	27,150	23,110
Liver and Intrahepatic Bile Duct	29,200	11,510	19,610	9,310
Pancreas	27,970	25,700	22,300	20,790
Melanoma of the Skin	52,170	34,940	6,380	3,350
Urinary Bladder	60,490	18,540	12,240	4,630
Brain and Other Nervous System	13,450	10,350	9,620	7,080
Lymphoma	44,730	35,770	12,080	9,130
Myeloma	17,490	12,790	6,660	5,930
Leukemia	36,290	25,840	14,300	10,200
Breast	2,470	252,710	460	40,610
Prostate	161,360	—	26,730	—

cancer is the third leading cause of death [2, 3]. Stomach cancer in males and breast cancer in females are the most prevalent cancers in Iran according to data from the GLOBOCAN in 2012[4]. Table 2 shows the number of deaths of various cancers and its percentage of total deaths in Iran and its ranking in the world. The information is based on the World Health Organization (WHO) data published in 2017 in Iran. According to Table 2, stomach cancer is the most common cause of cancer death in Iran, ranking 14th in the world.

Table 2: Number of Cancer Deaths and Iran Ranking in the World in 2017 Based on WHO Report

	Total Deaths, No. (%)	World Rank
Stomach Cancer	8850 (2.73)	14
Esophagus Cancer	5303 (1.63)	19
Lung	4816 (1.48)	106
Colon and Rectum Cancers	4691 (1.45)	93
Breast Cancer	3545 (1.09)	152
Lymphomas	3498 (1.08)	73
Leukemia	3240 (1.00)	24
Prostate Cancer	2483 (0.77)	137
Bladder Cancer	2475 (0.76)	16
Liver Cancer	1563 (0.48)	166
Pancreas Cancer	1194 (0.37)	124
Ovary Cancer	1179 (0.36)	116
Oral Cancer	855 (0.26)	157
Cervical Cancer	390 (0.12)	174
Skin Cancers	405 (0.12)	143
Uterine Cancer	210 (0.06)	170

Cancer cells can migrate through the bloodstream, lymph vessels, or tissue from one organ to another, which is called the metastasis process. Cancer deaths are also due to damage of other organs caused by the metastasis. Damage to the DNA that cannot be repaired by the body can cause cancer cells. The damaged DNA can be inherited or caused by external factors such as exposure to UV radiation, alcohol and tobacco, low physical activity, and exposure to dangerous chemicals and viruses such as hepatitis B and C [5, 6].

Surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy are common cancer treatment methods. Surgery is an invasive procedure by excision and removing the tumor and tissue around it and in some cases regional lymph nodes. The spread of cancer cells through metastases is the main cause of the limitation of this treatment [5]. In radiotherapy method, radiations such as gamma, X-ray, and proton are used to kill cancer cells. According to the type of cancer, internal or external radiotherapy is used for treatment. The side effects of irradiation in radiotherapy need further investigation [7]. In the case of cancers such as breast and prostate, the growth of cancer cells is hormone-dependent; therefore, hormone therapy can be used to treat cancer. Based on this method, the natural hormone cannot reach the target cells due to the use of inhibiting hormones. However, interference with the behavior of hormones has unwanted side effects in the body [8, 9]. In immunotherapy, the immune system is used to kill cancer cells. In this method, agents are used to stimulating or strengthening the

immune system to identify and kill cancer cells. This method also has side effects; for example, the accumulation of defense cells in the body can damage the nervous system [10]. The most common cancer treatment method is chemotherapy, which uses toxic drugs to kill cancer cells. Although chemotherapy is the best method to treat cancer, it also has adverse side effects due to the non-specificity of anticancer drugs. Nausea and vomiting, hair loss, reduced red and white blood cells and feeling tired due to the outcomes of chemotherapy [5, 11].

Scientists are now looking for approaches to reduce the side effects of cancer treatment by targeting cancer cells. For example, designing targeted carriers that can deliver anticancer drugs to cancer cells is a strategy that can reduce the side effects of chemotherapy. Drug delivery can be performed through a variety of methods such as oral, parenteral, mucosal, and transdermal drug delivery. According to the advantages of each of the different drug delivery methods and considering the type of disease and treatment location, one of them can be employed for more effective treatment [12]. For example, oral drug delivery is a non-invasive and most convenient method for the administration of a drug accepted by patients more than other methods, but it has low bioavailability [13, 14]. In contrast, parenteral drug delivery is an invasive injection method, but has high bioavailability, resulting in faster treatment [15, 16].

The most commonly employed method to treat cancer is parenteral drug delivery, and researchers are seeking to design carriers with prolonged release to reduce the number of injections and targeted carriers to reduce side effects. The current review study aimed at describing the use of chitosan and graphene in cancer treatment, and furthermore, considering the advantage of combining these two substances, chitosan-graphene nanocomposite was introduced as a pH-sensitive carrier for cancer theragnosis. Figure 1 shows a scheme for the use of pH-sensitive nanocomposite based on graphene-grafted chitosan for cancer theragnosis.

Chitosan

Chitosan cationic polysaccharide has the ideal potential to be used in therapeutic applications due to its unique biological properties. Biocompatibility, biodegradability, and nontoxicity are one of the most critical features of chitosan's natural polymer, which make it an appropriate carrier to deliver various drugs that can release the therapeutic agents in a controlled and sustained manner. The presence of hydroxyl and amine groups in the chitosan structure

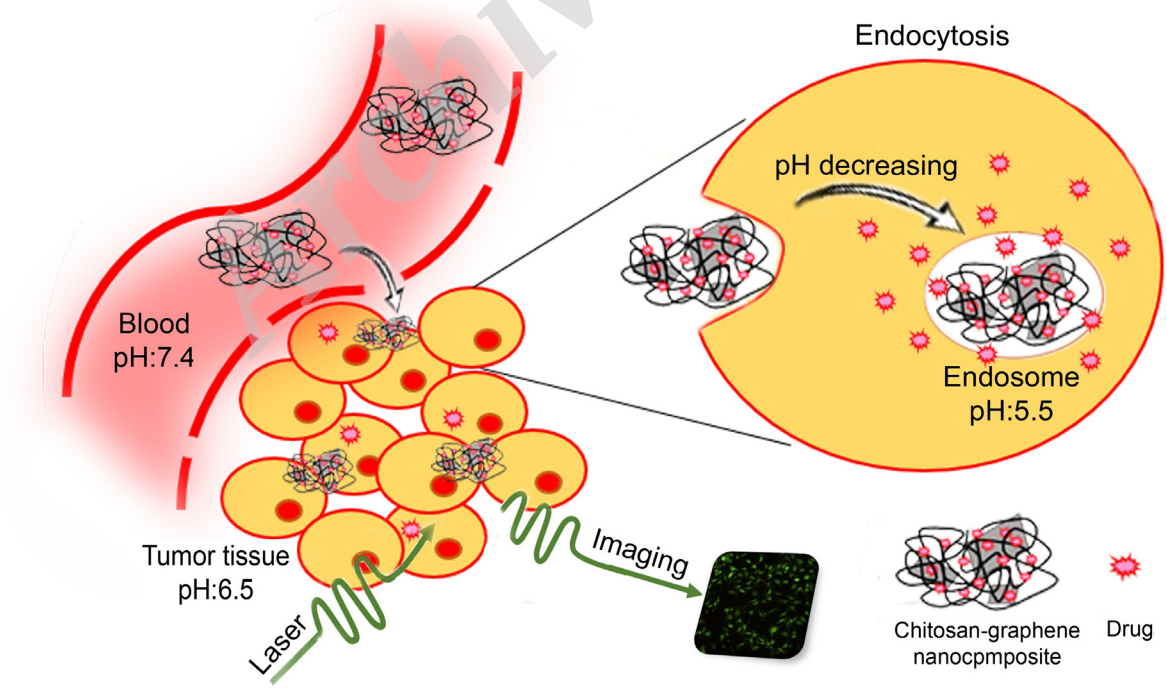

Figure 1: Schematic for the Use of Ph-Sensitive Nanocomposite Based on Graphene-Grafted Chitosan for Cancer Theragnosiss

provides the chemical modification that can help improve the solubility of chitosan carriers, interact chitosan with hydrophobic and hydrophobic drugs, and increase drug loading capacity [17, 18]. In addition to the expressed characteristics, due to the immunological and anticancer activity of chitosan, chitosan-based carriers can be a promising vehicle for anticancer drugs that attract a lot of attention [19, 20]. In 1984, Nishimura et al. showed the effect of activation of macrophages by chitosan and thus for the first time reported immunological activity of chitosan [21]. Increased immunity by chitosan can help to inhibit tumor growth, and therefore chitosan has an anticancer activity per se related to arresting the G1/S cell cycle and inducing apoptosis by increasing Caspase-3 activity. The positive charge of chitosan due to the amine groups in its body causes direct chitosan attachment to cancer cells with negative membranes. Chitosan, therefore, has cytotoxic effects on cancer cells, but not on non-cancerous cells [22, 23].

Chitosan Nanoparticle

Nanosized carriers create unique physico-chemical and biological properties in comparison to the bulk form. Small size and an increase in the surface-to-volume ratio of nanocarriers lead to increased solubility and bioavailability, longer circulation time by reducing the absorption by the reticuloendothelial system, and increasing the interactions of the carrier with the membrane of the cells. Chitosan nanoparticles prepared by various methods are widely used for drug delivery purposes. Chitosan nanocarrier can encapsulate a variety of anticancer drugs and target the site of the tumor, and thus creates an exciting potential for cancer treatment [24, 25]. For the first time in 1994, chitosan nanoparticles were prepared by Ohya et al., and the release of the 5-fluorouracil anticancer drug from this nanocarrier was investigated [26]. Chitosan nanoparticles with different particle sizes exhibit different inhibitor in tumor growth. Qi et al., obtained a 34.91%, 58.98%, and 61.69% tumor inhibition for three sizes of 40, 70, and 100 nm nanoparticles, respectively. The results indicated that tumor inhibition increases with particle size reduction, which is associated with better interactions of smaller particles with cell membranes [27]. The effect of EPR (enhanced permeability and retention) related to the physiological differences in tumor tissue and normal tissue, causes the accumulation of nanoparticles in the tumor tissue and passive targeting. Following the angiogenic process used to supply food and oxygen to the tumor tissue, leaky, fenestrated, and irregular blood vessels with poor lymphatic systems are formed. Therefore, particles of 10-200 nm can penetrate these defective vessels and remain in the tumor tissue, which is called the EPR effect [28, 29]. Mitra et al., synthesized chitosan nanoparticles with a size of 100 nm by microemulsion method. Anticancer drug doxorubicin conjugated with dextran was loaded in chitosan nanoparticles with 60%-65% encapsulation efficiency. The conjugate of doxorubicin with macromolecules dextran reduces the side effects of free doxorubicin. Therefore, chitosan nanoparticles were introduced as a carrier to dextran-doxorubicin for chemotherapy [30].

In a study in 2017, spherical chitosan nanoparticles loaded with carboplatin anticancer drug were used to treat breast cancer. The nanoparticles were prepared by ionic gelation method and had a size of 277 nm and a zeta potential of +31 mV. The encapsulation efficiency was 58.43, and load efficiency was 13.27 for carboplatin. The toxicity study by MTT assay in the MCF-7 cell line showed that chitosan nanoparticles did not show significant toxicity at concentrations of 200 g/mL at 24, 48, and 72 h [31].

Stimuli-Responsive Chitosan Carrier

The ultimate goal of designing drug delivery systems is to reduce side effects and improve treatment by targeting the intended site. Stimuli-responsive carriers are the strategy that helps with drug release on the target site. Various types of carriers that can respond to physical, chemical, and biological stimuli such as temperature, light, magnetism, pH, redox, and enzymes are used to design such drug delivery systems. Due to the difference in the pH value in normal and cancerous tissues, pH-sensitive drug delivery systems have a great advantage in treating cancer [32, 33]. The conversion of glucose to lactose, which occurs as a result of insufficient oxygen in the tumor tissue, results in a tumor tissue of pH 6.5-7.2, which is more acidic than the healthy tissue (pH 7.4). Also, after the uptake of drug carrier by cells through the endosomal pathway, it reaches endosomes (pH 5.5) and lysozymes (pH 4.5) [34, 35].

The presence of amine groups in chitosan converts it into a pH-sensitive polymer that can respond to pH changes. Chitosan is insoluble in neutral and alkaline environments, but it is soluble in acidic

environments due to the conversion of NH_2 to NH_3^+ groups. Chitosan pH-dependent behavior makes it an appropriate carrier to release cancer drugs that can reduce the side effects of anticancer drugs [29, 36].

Chitosan-Mediated Tumor-Targeted Drug Delivery System

The interaction between the carrier and the target cells results in the release of the drug in the predetermined site and active targeting. Different expression levels of specific markers on the surface of the target and non-target cells lead to their differentiation and targeted drug delivery. Some antigens and receptors are overexpressed in the cancer cell membrane. Therefore, chemical modification of carriers with ligands that interact with receptors on the surface of cancer cells leads to the detection of cancer cells and the occurrence of active targeting [28, 37]. Folate, hyaluronic acid (HA), and transferrin are some of the ligands studied for targeted drug delivery. For example, folate receptors are overexpressed at the epithelial surface of many types of cancers including the brain, kidney, colon, lung, and breast. Therefore, binding of the folic acid to the carrier leads to the high affinity with cell membrane folate receptor and increased endocytosis [38]. The amine and hydroxyl groups in chitosan provide chemical modification of chitosan with ligands to interact with the receptor ligand. For example, the reaction between amine chitosan and carboxylic acid folate leads to the formation of the chitosan-folate complex. For example, Wang et al., developed chitosan-folate nanoparticles and investigated their cellular uptake in HT-29 cell cancer cells to improve colon cancer [39]. In another study, the release of the anticancer drug, doxorubicin, from magnetic nanoparticles coated with folate-grafted chitosan was investigated. In this work, folate ligand was used to reduce the side effects of doxorubicin [40].

Graphene

Graphene can be considered as a platform for drug delivery purposes concerning the high surface area and functional groups existing on its surface. The π-π stacking of aromatic drug with graphene conjugated structure can lead to high loading of anticancer drugs such as doxorubicin, paclitaxel, camptothecin, and epirubicin [41]. For example, Yang et al. studied the loading of doxorubicin hydrochloride on graphene oxide based on the π-π stacking between drug and graphene. They showed that by increasing the initial drug, the drug loading rate increased linearly [42].

The functional groups in graphene also bind

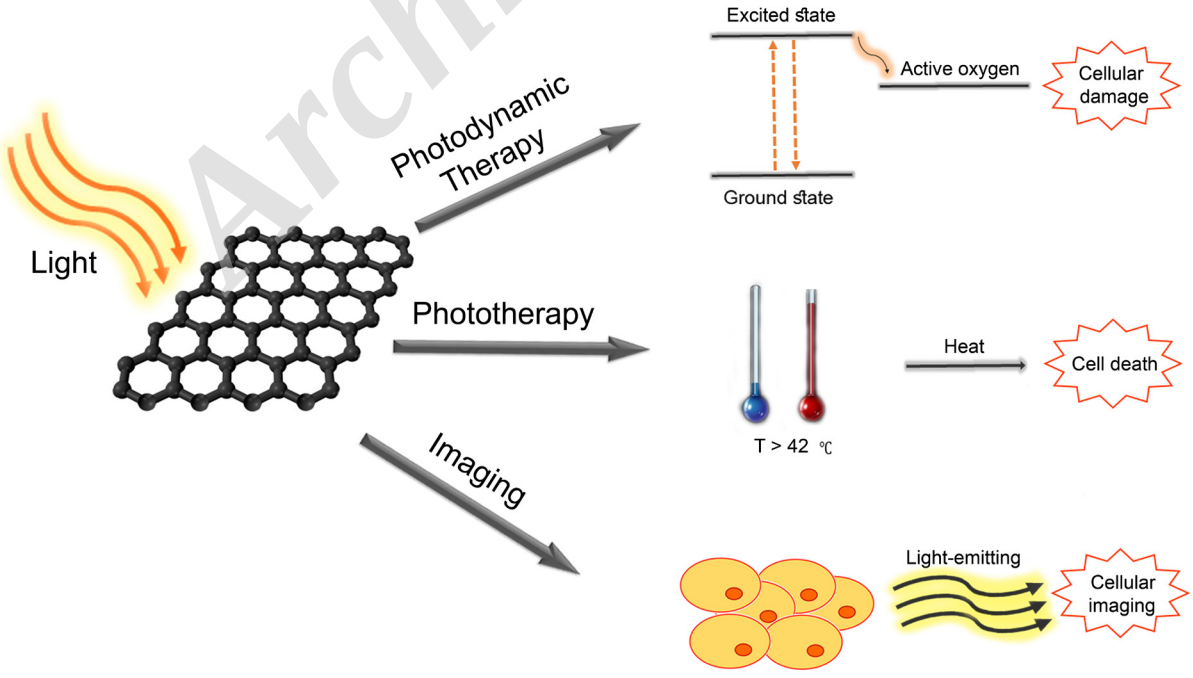

Figure 2: Application of Graphene in Photodynamic Therapy, Phototherapy, and Imaging

target molecules to graphene sheets for targeted drug delivery. For example, the formation of the amide bond between amine groups of folic acid and carboxylic acid groups of graphene oxide leads to ligand binding of the target folate, which is mostly expressed in cancerous cells [43]. In another study, the carrier of graphene/HA was prepared for targeted drug delivery, and the carrier was used to load epirubicin using π-π interactions [44]. According to the extraordinary optical, thermal, and electrical properties of graphene, it nowadays applied to diagnostic and imaging purposes as well as photodynamic therapy and phototherapy for cancer treatment. Figure 2 shows the application of graphene in photodynamic therapy, phototherapy, and imaging, which is explained below.

Photodynamic Therapy

Photodynamic therapy is a phototherapy method used in cancer treatment and reduces damage to healthy tissue due to lack of radiation to adjacent tissues. In this method, cancerous tissue is degraded by reaction with active oxygen species such as free radicals and singlet oxygen. They are activated by the light exposure to a photosensitive agent, and transfer of energy from the excited state to a ground state, which leads to the production of reactive oxygen species. Following the light exposure to photosensitizer, they are activated, and transfer of energy from an excited state to ground state leads to the production of reactive oxygen species. Therefore, photosensitizer has a prominent role in effective photodynamic therapy. Porphyrins, phthalocyanines, bacteriochlorins, and chlorins are the photosensitizers that already have been investigated [45, 46]. The employment of vehicles for delivery of photosensitizers is a technique that helps to provide photosensitizers with better solubility and selectivity. Graphene can be proposed as an ideal photosensitizer carrier that provides photodynamic therapy with other therapies, such as chemotherapy.

Zhou et al., used graphene oxide to load anti-cancer drugs SN-38 and hypocrellin A as photosensitive agents and studied the potential of this system for photodynamic therapy and chemotherapy simultaneously [47]. In another study, binding the target ligand (folic acid) to graphene oxide loaded with photosensitizer chlorin e6 led to more accumulation of this system in the tumor tissue and resulted in targeted photodynamics [48]. Also, in a study showed that graphene quantum dots due to the MSS (multistate sensitization) mechanism had higher $^1O_2$ quantum yields than conventional photosensitizers such as porphyrin, and therefore, graphene quantum dot can be introduced as a photosensitizer alone [49].

Phototherapy

By increasing the temperature of the cancerous tissue to more than 42°C, the cancer cells die. Photothermal agents by absorbing light and converting it to heat lead to local hyperthermia and destroy cancer cells. In photothermal therapy, preferably near-infrared (NIR) light is used and due to increasing its penetration in the tissue leads to less damage; as well as tissue is transparent in NIR light, which can also be combined imaging with the treatment [50]. Therefore, materials with high absorbance values in the NIR range can be used as photothermal agents in phototherapy. Due to the absorption of light from the UV to the NIR by graphene, its application in phototherapy is widely considered. When NIR light is radiated on graphene, the electron is transferred from the ground state to the excited state and vibration of such electrons leads to heat generation and the destruction of cancer cells [51].

Graphene, due to its large surface area and high drug loading capacity, can provide a combination set of chemotherapy and phototherapy, which is a strategy to reduce drug and multidrug resistance. Tran et al. loaded doxorubicin and irinotecan onto graphene and studied the combination of chemotherapy and phototherapy [52]. Loading a photosensitizer onto the surface of graphene oxide can provide a combined set of photodynamic and phototherapy. Sahu et al., synthesized methylene blue-loaded nanographene oxide by electrostatic interaction between the negative charge of graphene oxide and a positive charge of methylene. Methylene blue is used as a photosensitizer with high $^1O_2$ quantum yield in photodynamics. The faster release of methylene blue in acidic environments due to the reduction of the electrostatic power between nanographene oxide and methylene blue indicated the pH-dependent release of this system [53].

Imaging

Quantum dots can emit light due to the quantum confinement; this quantum dot luminescence feature is used for biological imaging. The bandgap in the

zero-dimensional graphene and the presence of structural defects are two reasons for the fluorescence properties of nanosized graphene. Since tissue is transparent at NIR wavelengths, the use of this wavelengths is widely considered for biological imaging and cancer diagnosis purposes [54, 55]. Sun et al., with the covalent grafting of polyethylene glycol to nano-graphene oxide, produced pegylated nano-graphene oxide to improve solubility in biological solutions. By the employment of π-π stacking, they loaded doxorubicin onto graphene. Due to the fluorescence properties of nano-graphene oxide, the system simultaneously used drug delivery and imaging [56].

According to Raman signals of graphene oxide, it can be used as a Raman probe for cell and tissue imaging. The weak Raman signal of graphene oxide can be optimized using surface-enhanced Raman scattering labels. The preparation of graphene oxide-metal nanoparticle hybrid contributes to surface-enhanced Raman scattering effect [57]. Liu et al., produced hybrid graphene oxide-silver nanoparticles for fast SERS (surface-enhanced Raman scattering) imaging (0.06 s per pixel). Targeted SERS imaging was achieved by the binding folic acid ligand to graphene oxide [58]. In a study, the Au@NGO (graphene oxide wrapped gold nanoparticles) hybrid was synthesized, and doxorubicin loaded onto the surface of the nano-graphene oxide and used as the hybrid for drug delivery and Raman imaging [59].

Chitosan-Graphene

According to the expressed features, the application of chitosan and graphene to treat cancer has raised the attention of researchers. According to Figure 3, the number of articles published in this field is increasing. Data were obtained by searching the keywords "chitosan and cancer" and "graphene and cancer" in the Scopus database.

The chitosan-graphene nanocomposite, utilizing the benefits of both graphene and chitosan substances, provides ideal multipurpose carriers for cancer treatment [12]. Adding graphene with high Young's modulus to chitosan helps to improve the mechanical properties of chitosan. The poor mechanical strength of chitosan, which is one of the problems with the use of chitosan in drug delivery, can be improved by adding graphene as a filler to reinforce the matrix [60, 61]. Justin et al. found that the presence of graphene sheets reduced the chitosan biodegradability rate [60]. Also, according to the application of graphene in photodynamic therapy, phototherapy, and imaging, as described above, the introduction of graphene into chitosan-based carriers

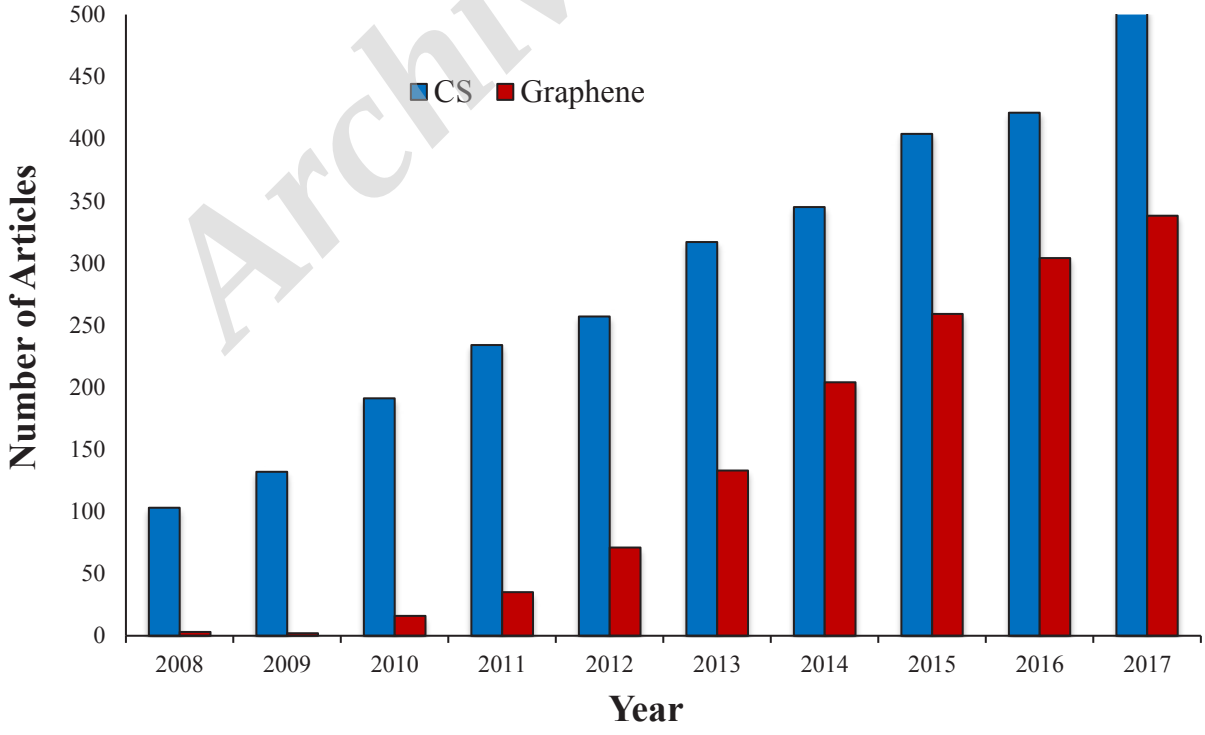

Figure 3: The Number of Published Articles From 2008 to 2017

makes it possible to use such therapeutic methods and diagnostic simultaneously with chemotherapy in graphene-grafted chitosan composites, which contribute to effective cancer treatment.

Considering the importance of the toxicity of materials used in biomedical systems and evaluating the toxicity of carbon-based materials, dose-dependent cytotoxicity of graphene is confirmed [62, 63]. Therefore, the combination of graphene with biocompatible materials such as natural polymers can reduce toxicity and improve the biocompatibility of graphene-based carriers. Due to the unique properties of chitosan in the treatment of cancer, especially its pH-sensitiveness, chitosan-graphene nanocomposite can be an ideal carrier for the targeted release of cancer drugs. In 2011, for the first time, the chitosan/graphene oxide composite was synthetized by forming an amide bond between the carboxylic acid group of graphene oxide and the amine group of chitosan. This new carrier was used to load and release the anti-cancer drug CPT (camptothecin). The release of CPT in phosphate buffered saline (PBS) shows that about 17.5% of the drug is released upon 72 hr. MTT assay results showed that the cell viability of the new carrier is more than 80%[64].

In 2016, carboxymethyl chitosan/graphene carrier was synthesized to deliver anticancer drug doxorubicin. The loading of doxorubicin onto graphene was achieved by the π-π stacking. Carboxymethyl chitosan, a biocompatible derivative of chitosan with better solubility, serves as a mediator to bind target ligand of HA and fluorescein isothiocyanate and provides targeted drug delivery. The release of the drug at pH values 5.8 and 7.4 showed the pH-sensitiveness of the GO-CMC-FI-HA composite [65]. Wang et al., developed the chitosan/graphene nanogel as a platform for remote controlling anticancer drugs delivery by an external stimulant. In their study, graphene was used as a photosensitizer to convert light into heat and used chitosan as a thermo-sensitive hydrogel that changes its swelling with the temperature. Increasing the temperature through the photothermal property of graphene by absorbing NIR light leads to shrinking chitosan hydrogel and releasing the drug. Doxorubicin was loaded onto the carrier as a drug model; the drug loading increased with increasing initial concentrations. The study on the release of doxorubicin at 37°C and 42°C showed a faster release of the drug from the carrier at 42°C, which confirmed the thermosensitivity of this platform. Evaluation of NIR-triggered anticancer drug delivery showed that after exposing the tissue to laser in five cycles (three minutes irradiation with 30 minutes interval for each cycle), 18.81% of the drug was released from the carrier in comparison with 5% without radiation [66].

In a study, chitosan/graphene hybrid was used for targeted release and cellular imaging. In this work, doxorubicin was loaded onto graphene oxide through the π-π stacking and hydrogen bonding, and the loading efficiency was 87%. Also, cyclicRGD (cRG) peptides were linked to the chitosan as target ligand via thiolation reaction using SPDP (N-succinimidyl 3-(2-pyridyldithio)-propionate). Integrin receptors are expressed on the surface of the hepatocellular carcinoma cells; hence, due to the high cRG binding affinity with integrin receptors, cRG-modified chitosan can be used for targeted release in the treatment of liver cancer. The cRG-modified chitosan and drug-loaded graphene were prepared by the noncovalent method, and the drug release was assessed at pH values 5.5 and 7.4. The faster release in the acid environment shows the pH-sensitiveness of this carrier. The cellular uptake of FITC-labeled RC-GO carrier was investigated using a laser confocal fluorescence microscope (LCFM) [67].

Lv et al., introduced the graphene quantum dots-chitosan xerogels as drug carriers and imaging. The graphene quantum dots-chitosan xerogels were formed through electrostatic interactions between positive charge of chitosan and negative charge of graphene quantum dots, as well as hydrogen bonding between them. The sodium salicylate was loaded onto the carrier as the drug model; the amount of encapsulation efficiency was 30%, and the loading capacity was 13%. The protonation and deprotonation of the amine group of chitosan lead to the release of a pH-sensitive drug from the carrier. Due to the fluorescence emission property of graphene quantum dot, these carriers are used as fluorescent probes and in vivo imaging [68].

In a study, the magnetic chitosan/graphene oxide platform was synthesized for loading with 3-[1-hydroxyethyl]-3-devinyl-13^1-β,β-dicyanomethylene-13^1-deoxopyropheophorbide-a (HNPa) as a photosensitizer agent. This photosensitizer carrier provided good stability and solubility and magnetic targeting for photodynamic

treatment. The loading capacity of HNPa with an initial concentration of 1 $mg.mL^{-1}$ was 57.6%, and the release profile showed a faster release of HNPa in acidic environments compared to physiological conditions. The results of produced singlet oxygen in comparison with the methylene blue standard showed that the $^1O_2$ quantum yield of the carrier loaded with HNPa (62.9%) was higher than that of the HNPa alone (42.6%). Therefore, the magnetic chitosan/graphene oxide composite improves photodynamic therapy by increasing the produced singlet oxygen [69].

In a study by Zhao et al., were synthesized graphene oxide nanoparticle/chitosan hybrids were used as a pH-sensitive carrier for anticancer drugs. The chitosan/dimethylmaleic anhydride-modified chitosan, as an outer shell, give the charge-reversibility property to the carrier, which provides a negative charge to the carrier in the blood circulation leading to its stability, and positive charge in the tumor environment leading to increased cellular uptake of the carrier. The core of graphene oxide nanoparticles with an aromatic structure leads to doxorubicin loading with an encapsulated yield of 89.35% and a loading capacity of 0.8935 mg/mg. The release of the drug at pH values 5, 6.5, and 7 shows the pH-dependent release of this hybrid [70].

CONCLUSION

Chitosan has unique biological properties such as biocompatibility, biodegradability, anticancer activity and the ability to pass chitosan nanoparticles from irregular blood vessels based on the EPR phenomenon. Creating stimuli-responsive carriers specially pH-sensitive and the design of vehicles with targeted ligands for targeting drug delivery led to the introduction of chitosan-based carriers as the ideal candidate for cancer treatment, which highly considered in recent years. The large surface area and the aromatic structure of graphene provide high loading anticancer drug for chemotherapy. Also, due to its unique structural characteristics, graphene can be used in the other methods of cancer treatment such as photodynamic therapy, phototherapy, and in vivo imaging. Therefore, chitosan and graphene hybrids can be possibly employed to design multipurpose carriers with several therapeutic approaches such as chemotherapy, phototherapy, and photodynamic therapy as well as imaging to diagnose and treat cancer simultaneously.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICS APPROVAL

The current study was approved by the Ethics Committee of Research Laboratory of Green Organic Synthesis and Polymers, Department of Chemistry, Iran University of Science and Technology (IUST).

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017;67(1):7-30. DOI: 10.3322/caac.21387 PMID: 28055103
2. Mohammadian M, Salehiniya H, Mohammadian-Hafshejani A. Some facts on incidence and mortality of cancer in Iran. Iranian Journal of Public Health. 2017;46(10):1446-7. PMID: 29308393
3. Radmard AR. Five common cancers in Iran. Archives of Iranian medicine. 2010;13(2):143. PMID: 20187669
4. Amirkhah R, Naderi-Meshkin H, Mirahmadi M, Allahyari A, Sharifi HR. Cancer statistics in Iran: Towards finding priority for prevention and treatment. Cancer Press. 2017;3(2):27-38. DOI: 10.15562/tcp.42
5. Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. J Cancer Sci Ther. 2009;1(2):1-4. DOI: 10.4172/1948-5956.100000e2 PMID: 20740081
6. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research. 2008;25(9):2097-116. DOI: 10.1007%2Fs11095-008-9661-9 PMID: 18626751
7. Delaney G, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. Cancer. 2005;104(6):1129-37. DOI: 10.1002/cncr.21324 PMID: 16080176
8. Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. The Lancet. 2003;362(9382):419-27. DOI: 10.1016/S0140-6736(03)14065-2
9. Byar DP, Corle DK. Hormone therapy for prostate cancer: results of the Veterans Administration Cooperative Urological Research Group studies. NCI Monogr. 1988(7):165-70. PMID: 3050535
10. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immu-

notherapy for cancer: harnessing the T cell response. Nat Rev Immunol. 2012;12(4):269-81. DOI: 10.1038/nri3191 PMID: 22437939

11. Iwamoto T. Clinical application of drug delivery systems in cancer chemotherapy: review of the efficacy and side effects of approved drugs. Biol Pharm Bull. 2013;36(5):715-8. DOI: 10.1248/bpb.b12-01102 PMID: 23649331
12. Gooneh-Farahani S, Naimi-Jamal MR, Naghib SM. Stimuli-responsive graphene-incorporated multifunctional chitosan for drug delivery applications: a review. Expert opinion on drug delivery. 2019([e-pub ahead of print]).
13. Kaur R, Bala R, Malik D. A novel approach in oral fast dissolving drug delivery system–a review. American Journal of PharmTech Research. 2012;2(1):88-104.
14. Moroz E, Matoori S, Leroux J-C. Oral delivery of macromolecular drugs: Where we are after almost 100 years of attempts. Advanced drug delivery reviews. 2016;101:108-21. DOI: 10.1016/j.addr.2016.01.010 PMID: 26826437
15. Shi Y, Li LC. Current advances in sustained-release systems for parenteral drug delivery. Expert Opin Drug Deliv. 2005;2(6):1039-58. DOI: 10.1517/17425247.2.6.1039 PMID: 16296808
16. Larsen C, Larsen SW, Jensen H, Yaghmur A, Ostergaard J. Role of in vitro release models in formulation development and quality control of parenteral depots. Expert Opin Drug Deliv. 2009;6(12):1283-95. DOI: 10.1517/17425240903307431 PMID: 19941410
17. Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev. 2010;62(1):3-11. DOI: 10.1016/j.addr.2009.09.004 PMID: 19800377
18. Prabaharan M. Chitosan derivatives as promising materials for controlled drug delivery. Journal of biomaterials applications. 2008;23(1):5-36. DOI: 10.1177/0885328208091562 PMID: 18593819
19. Dass CR, Choong PF. The use of chitosan formulations in cancer therapy. Journal of microencapsulation. 2008;25(4):275-9. DOI: 10.1080/02652040801970461 PMID: 18465306
20. Xia W, Liu P, Zhang J, Chen J. Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloids. 2011;25(2):170-9. DOI: 10.1016/j.foodhyd.2010.03.003
21. Nishimura K, Nishimura S, Nishi N, Saiki I, Tokura S, Azuma I. Immunological activity of chitin and its derivatives. Vaccine. 1984;2(1):93-9. DOI: 10.1016/S0264-410X(98)90039-1 PMID: 6397928
22. Wimardhani YS, Suniarti DF, Freisleben HJ, Wanandi SI, Siregar NC, Ikeda M-A. Chitosan exerts anticancer activity through induction of apoptosis and cell cycle arrest in oral cancer cells. Journal of oral science. 2014;56(2):119-26. DOI: 10.2334/josnusd.56.119 PMID: 24930748
23. Hasegawa M, Yagi K, Iwakawa S, Hirai M. Chitosan induces apoptosis via caspase-3 activation in bladder tumor cells. Jpn J Cancer Res. 2001;92(4):459-66. PMID: 11346469
24. Qi L, Xu Z. In vivo antitumor activity of chitosan nanoparticles. Bioorg Med Chem Lett. 2006;16(16):4243-5. DOI: 10.1016/j.bmcl.2006.05.078 PMID: 16759859
25. Prabaharan M. Chitosan-based nanoparticles for tumor-targeted drug delivery. Int J Biol Macromol. 2015;72:1313-22. DOI: 10.1016/j.ijbiomac.2014.10.052 PMID: 25450550
26. Ohya Y, Shiratani M, Kobayashi H, Ouchi T. Release behavior of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. Journal of Macromolecular Science—Pure and Applied Chemistry. 1994;31(5):629-42. DOI: 10.1080/10601329409349743
27. Qi L, Xu Z, Chen M. In vitro and in vivo suppression of hepatocellular carcinoma growth by chitosan nanoparticles. Eur J Cancer. 2007;43(1):184-93. DOI: 10.1016/j.ejca.2006.08.029 PMID: 17049839
28. Bae YH, Park K. Targeted drug delivery to tumors: myths, reality and possibility. J Control Release. 2011;153(3):198-205. DOI: 10.1016/j.jconrel.2011.06.001 PMID: 21663778
29. Ghaz-Jahanian MA, Abbaspour-Aghdam F, Anarjan N, Berenjian A, Jafarizadeh-Malmiri H. Application of chitosan-based nanocarriers in tumor-targeted drug delivery. Mol Biotechnol. 2015;57(3):201-18. DOI: 10.1007/s12033-014-9816-3 PMID: 25385004
30. Mitra S, Gaur U, Ghosh P, Maitra A. Tumour targeted delivery of encapsulated dextran–doxorubicin conjugate using chitosan nanoparticles as carrier. Journal of Controlled Release. 2001;74(1-3):317-23. DOI: 10.1016/S0168-3659(01)00342-X
31. Khan MA, Zafaryab M, Mehdi SH, Quadri J, Rizvi MM. Characterization and carboplatin loaded chitosan nanoparticles for the chemotherapy against breast cancer in vitro studies. Int J Biol Macromol. 2017;97:115-22. DOI: 10.1016/j.ijbiomac.2016.12.090 PMID: 28082219
32. Liu J, Huang Y, Kumar A, Tan A, Jin S, Mozhi A, et al. pH-sensitive nano-systems for drug delivery in cancer therapy. Biotechnol Adv. 2014;32(4):693-710. DOI: 10.1016/j.biotechadv.2013.11.009 PMID: 24309541
33. Yin Q, Shen J, Zhang Z, Yu H, Li Y. Reversal of multidrug resistance by stimuli-responsive drug delivery systems for therapy of tumor. Adv Drug Deliv Rev. 2013;65(13-14):1699-715. DOI: 10.1016/j.addr.2013.04.011 PMID: 23611952
34. Hu YB, Dammer EB, Ren RJ, Wang G. The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. Transl Neurodegener. 2015;4(1):18. DOI: 10.1186/s40035-015-0041-1 PMID: 26448863
35. Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. Adv Drug Deliv Rev. 2006;58(15):1655-70. DOI: 10.1016/j.addr.2006.09.020 PMID: 17125884
36. Suarato G, Li W, Meng Y. Role of p H-responsiveness in the design of chitosan-based cancer nanotherapeutics: a review. Biointerphases. 2016;11(4):04B201.
37. Manish G, Vimukta S. Targeted drug delivery system: a review. Res J Chem Sci. 2011;1(2):135-8.
38. Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev. 2000;41(2):147-62. DOI: 10.1016/S0169-409X(99)00062-9 PMID: 10699311
39. Li P, Wang Y, Zeng F, Chen L, Peng Z, Kong LX. Synthesis and characterization of folate conjugated chitosan and cellular uptake of its nanoparticles in HT-29 cells. Carbohydr Res. 2011;346(6):801-6. DOI: 10.1016/j.carres.2011.01.027 PMID: 21397214

40. Yang CL, Chen JP, Wei KC, Chen JY, Huang CW, Liao ZX. Release of Doxorubicin by a Folate-Grafted, Chitosan-Coated Magnetic Nanoparticle. Nanomaterials (Basel). 2017;7(4):85. DOI: 10.3390/nano7040085 PMID: 28406429
41. Liu J, Cui L, Losic D. Graphene and graphene oxide as new nanocarriers for drug delivery applications. Acta biomaterialia. 2013;9(12):9243-57. DOI: 10.1016/j.actbio.2013.08.016 PMID: 23958782
42. Yang X, Zhang X, Liu Z, Ma Y, Huang Y, Chen Y. High-efficiency loading and controlled release of doxorubicin hydrochloride on graphene oxide. The Journal of Physical Chemistry C. 2008;112(45):17554-8. DOI: 10.1021/jp806751k PMID: 28406429
43. Qin XC, Guo ZY, Liu ZM, Zhang W, Wan MM, Yang BW. Folic acid-conjugated graphene oxide for cancer targeted chemo-photothermal therapy. J Photochem Photobiol B. 2013;120:156-62. DOI: 10.1016/j.jphotobiol.2012.12.005 PMID: 23357205
44. Jung HS, Lee M-Y, Kong WH, Do IH, Hahn SK. Nano graphene oxide–hyaluronic acid conjugate for target specific cancer drug delivery. RSC Advances. 2014;4(27):14197-200. DOI: 10.1039/c4ra00605d
45. Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. Nat Rev Cancer. 2003;3(5):380-7. DOI: 10.1038/nrc1071 PMID: 12724736
46. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, et al. Photodynamic therapy. J Natl Cancer Inst. 1998;90(12):889-905. DOI: 10.1093/jnci/90.12.889 PMID: 9637138
47. Zhou L, Wei S, Ge X, Zhou J, Jiang H, Li F, et al. Combination of chemotherapy and photodynamic therapy using graphene oxide as drug delivery system. Journal of Photochemistry and Photobiology B: Biology. 2014;135:7-16. DOI: 10.1016/j.jphotobiol.2014.04.010 PMID: 24792568
48. Huang P, Xu C, Lin J, Wang C, Wang X, Zhang C, et al. Folic Acid-conjugated Graphene Oxide loaded with Photosensitizers for Targeting Photodynamic Therapy. Theranostics. 2011;1:240-50. DOI: 10.7150/thno/v01p0240 PMID: 21562631
49. Ge J, Lan M, Zhou B, Liu W, Guo L, Wang H, et al. A graphene quantum dot photodynamic therapy agent with high singlet oxygen generation. Nat Commun. 2014;5:4596. DOI: 10.1038/ncomms5596 PMID: 25105845
50. Goodarzi S, Mozafari M. Phototherapy Techniques for Cancer Diagnosis and Treatment. 2015.
51. Nurunnabi M, Khatun Z, Reeck GR, Lee DY, Lee YK. Photoluminescent graphene nanoparticles for cancer phototherapy and imaging. ACS Appl Mater Interfaces. 2014;6(15):12413-21. DOI: 10.1021/am504071z PMID: 25054687
52. Tran TH, Nguyen HT, Pham TT, Choi JY, Choi HG, Yong CS, et al. Development of a Graphene Oxide Nanocarrier for Dual-Drug Chemo-phototherapy to Overcome Drug Resistance in Cancer. ACS Appl Mater Interfaces. 2015;7(51):28647-55. DOI: 10.1021/acsami.5b10426 PMID: 26641922
53. Sahu A, Choi WI, Lee JH, Tae G. Graphene oxide mediated delivery of methylene blue for combined photodynamic and photothermal therapy. Biomaterials. 2013;34(26):6239-48. DOI: 10.1016/j.biomaterials.2013.04.066 PMID: 23706688
54. Li J-L, Tang B, Yuan B, Sun L, Wang X-G. A review of optical imaging and therapy using nanosized graphene and graphene oxide. Biomaterials. 2013;34(37):9519-34. DOI: 10.1016/j.biomaterials.2013.08.066 PMID: 24034502
55. Cao L, Meziani MJ, Sahu S, Sun YP. Photoluminescence properties of graphene versus other carbon nanomaterials. Acc Chem Res. 2013;46(1):171-80. DOI: 10.1021/ar300128j PMID: 23092181
56. Sun X, Liu Z, Welsher K, Robinson JT, Goodwin A, Zaric S, et al. Nano-Graphene Oxide for Cellular Imaging and Drug Delivery. Nano Res. 2008;1(3):203-12. DOI: 10.1007/s12274-008-8021-8 PMID: 20216934
57. Liu Q, Wei L, Wang J, Peng F, Luo D, Cui R, et al. Cell imaging by graphene oxide based on surface enhanced Raman scattering. Nanoscale. 2012;4(22):7084-9. DOI: 10.1039/c2nr32525j PMID: 23070130
58. Liu Z, Guo Z, Zhong H, Qin X, Wan M, Yang B. Graphene oxide based surface-enhanced Raman scattering probes for cancer cell imaging. Phys Chem Chem Phys. 2013;15(8):2961-6. DOI: 10.1039/c2cp43715e PMID: 23340832
59. Ma X, Qu Q, Zhao Y, Luo Z, Zhao Y, Ng KW, et al. Graphene oxide wrapped gold nanoparticles for intracellular Raman imaging and drug delivery. Journal of Materials Chemistry B. 2013;1(47):6495-500. DOI: 10.1039/c3tb21385d
60. Justin R, Chen B. Characterisation and drug release performance of biodegradable chitosan–graphene oxide nanocomposites. Carbohydrate polymers. 2014;103:70-80. DOI: 10.1016/j.carbpol.2013.12.012 PMID: 24528702
61. Pan Y, Wu T, Bao H, Li L. Green fabrication of chitosan films reinforced with parallel aligned graphene oxide. Carbohydrate polymers. 2011;83(4):1908-15. DOI: 10.1016/j.carbpol.2010.10.054
62. Zhang X, Hu W, Li J, Tao L, Wei Y. A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotubes, graphene oxide, and nanodiamond. Toxicology Research. 2012;1(1):62-8. DOI: 10.1039/c2tx20006f
63. Chang Y, Yang ST, Liu JH, Dong E, Wang Y, Cao A, et al. In vitro toxicity evaluation of graphene oxide on A549 cells. Toxicol Lett. 2011;200(3):201-10. DOI: 10.1016/j.toxlet.2010.11.016 PMID: 21130147
64. Bao H, Pan Y, Ping Y, Sahoo NG, Wu T, Li L, et al. Chitosan-functionalized graphene oxide as a nanocarrier for drug and gene delivery. Small. 2011;7(11):1569-78. DOI: 10.1002/smll.201100191 PMID: 21538871
65. Yang H, Bremner DH, Tao L, Li H, Hu J, Zhu L. Carboxymethyl chitosan-mediated synthesis of hyaluronic acid-targeted graphene oxide for cancer drug delivery. Carbohydr Polym. 2016;135:72-8. DOI: 10.1016/j.carbpol.2015.08.058 PMID: 26453853
66. Wang C, Mallela J, Garapati US, Ravi S, Chinnasamy V, Girard Y, et al. A chitosan-modified graphene nanogel for noninvasive controlled drug release. Nanomedicine.

2013;9(7):903-11. DOI: 10.1016/j.nano.2013.01.003 PMID: 23352802

67. Wang C, Chen B, Zou M, Cheng G. Cyclic RGD-modified chitosan/graphene oxide polymers for drug delivery and cellular imaging. Colloids Surf B Biointerfaces. 2014;122:332-40. DOI: 10.1016/j.colsurfb.2014.07.018 PMID: 25064484
68. Lv O, Tao Y, Qin Y, Chen C, Pan Y, Deng L, et al. Highly fluorescent and morphology-controllable graphene quantum dots-chitosan hybrid xerogels for in vivo imaging and pH-sensitive drug carrier. Mater Sci Eng C Mater Biol Appl. 2016;67:478-85. DOI: 10.1016/j.msec.2016.05.031 PMID: 27287145
69. Qin X, Zhang H, Wang Z, Jin Y. Magnetic chitosan/graphene oxide composite loaded with novel photosensitizer for enhanced photodynamic therapy. RSC Advances. 2018;8(19):10376-88. DOI: 10.1039/C8RA00747K
70. Zhao X, Wei Z, Zhao Z, Miao Y, Qiu Y, Yang W, et al. Design and Development of Graphene Oxide Nanoparticle/Chitosan Hybrids Showing pH-Sensitive Surface Charge-Reversible Ability for Efficient Intracellular Doxorubicin Delivery. ACS Appl Mater Interfaces. 2018;10(7):6608-17. DOI: 10.1021/acsami.7b16910 PMID: 29368916